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(19) (CA) **CANADIAN PATENT** (12)

(54) PROCESS FOR PREPARING CEPHALOSPORIN DERIVATIVES

(72) Shimizu, Bunji; Kaneko, Masakatsu;
Sugawara, Shinichi, Japan

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The present invention relates to a novel process for the preparation of certain 7β-acylamino-7α-methoxycephalosporin derivatives.

In one known process for preparing 7β-acylamino-7α-methoxycephalosporin derivatives, 7β-(ω-aminoadipoyl)amino-7α-methoxycephalosporin compounds are produced by fermentation and then acylated to form the corresponding diacylated compounds. The aminoadipoyl group is then split off (see Japanese Patent Provisional Publication No. 931/72). The same Japanese Patent Publication also describes a process in which a 7-amino-7-methoxycephalosporin compound obtained by chemical synthesis is acylated to give the corresponding 7β-acylamino-7α-methoxycephalosporin compound. In these processes, the acylation at the 7β-position is effected by the use of an active derivative of the acid whose residue it is desired to incorporate at the 7β-position, such as the acid chloride. If, however, the acyl group to be introduced is derived from a substituted thioalkanoic acid (such as 1,3,4-thiadiazolylthioacetic acid or 1,2,4-triazolylthioacetic), this process is not practical, since it is often difficult or, in some cases, impossible to prepare an active derivative of such an acid; in other cases, this active derivative may be unstable or may not

be obtainable in a good yield. In any case, the yield of the acylation reaction is extremely poor.

Another process is described in Japanese Patent Provisional Publication No. 62791/73. In this process, a cephalosporin compound, unsubstituted at the 7α- position, is converted to an acylimine intermediate, which is then methoxylated to give the desired 7α-methoxycephalosporin derivative. This process however, also has disadvantages in that it cannot be carried out economically and, in any case, still leaves unsolved the problem of introducing the desired 7β-acyl substituent in the starting material.

We have now discovered a process for the preparation of 7α-methoxycephalosporin derivatives having a substituted thioalkanoylamino group at the 7β- position, which enables these derivatives to be obtained under mild conditions, in very high yields compared with known processes and without any of the disadvantages described above. The process of the invention may be used to prepare a wide variety of cephalosporin derivatives.

Thus, in accordance with the present invention, there is provided a process for preparing a 7α-methoxycephalosporin

derivative having a 7β substituent of formula

$$R^1 - S - A - CO.NH-$$

(in which: R^1 represents a hydrogen atom, a substituted or unsubstituted acyclic hydrocarbon group, a substituted or unsubstituted monocyclic alicyclic hydrocarbon group having a 5- or 6-membered ring, a monocyclic or bicyclic aromatic group (whose benzene ring may be substituted or unsubstituted), a substituted or unsubstituted alkanoyl group, a substituted or unsubstituted benzoyl group, or a substituted or unsubstituted monocyclic or bicyclic heterocyclic group having at least one ring nitrogen, oxygen or sulphur atom; and A is a straight or branched-chain alkylene group), wherein a 7α-methoxycephalosporin derivative having a 7β substituent of formula

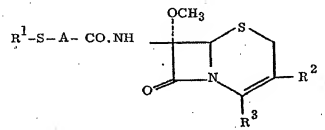
$$X - A - CO.NH-$$

(in which: A is as defined above; and X is a halogen atom) is reacted with a thiol compound of formula:

$$R^1 - SH \qquad (I)$$

or with a metal salt thereof.

The 7α-methoxycephalosporin derivative prepared is preferably a compound of formula (II):



(II)

(in which: R^1 is as defined above; R^2 is a group which does not participate in the reaction, such as those groups exemplified below; and R^3 is a carboxyl group or an esterified carboxyl group).

Examples of acyclic hydrocarbon groups which may be represented by R^1 are straight or branched- chain hydrocarbon groups having from 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, hexyl, vinyl, allyl, propenyl, butenyl, pentenyl, hexenyl, ethynyl or propargyl. These hydrocarbon groups may be unsubstituted or they may be substituted with such substituents as: hydroxy groups; azido groups; cyano groups; nitro groups; acylamino groups, e.g. acetylamino; alkoxycarbonylamino groups, such as as t-butoxycarbonylamino; alkoxy groups, e.g. methoxy or

ethoxy; the phenyl group; the cyclohexadienyl group; the cyclohexyl group; or alkoxycarbonyl groups, e.g. methoxycarbonyl or ethoxycarbonyl.

Where the group R^1 represents a monocyclic or bicyclic aromatic group, it may be, for example, a substituted or unsubstituted phenyl and naphthyl group. Examples of suitable substituents include, for example: halogen atoms, such as chlorine or bromine; alkyl groups, such as methyl or ethyl groups, alkoxy groups, such as methoxy or ethoxy; the cyano group; the nitro group; acylamino groups, such as acetylamino; or alkoxycarbonylamino groups, such as t-butoxycarbonylamino. If the group R^1 is a benzoyl group, it may be unsubstituted or substituted and, if substituted, the substituents may be any of those exemplified above with respect to the phenyl and naphthyl groups.

R^1 may also be a monocyclic alicyclic group having a 5- or 6-membered saturated or unsaturated ring; examples of such groups include: cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl and cyclohexadienyl.

Examples of alkanoyl groups, preferably having from 2 to 18 carbon atoms, which may be represented by

R^1 include acetyl, propionyl, butyroyl and stearoyl groups, which may be substituted or unsubstituted. If substituted, suitable substituents include: cyano groups; nitro groups; acylamino groups, e.g. acetylamino; alkoxycarbonylamino groups, such as t-butoxycarbonylamino; and alkoxycarbonyl groups, e.g. ethoxycarbonyl or methoxycarbonyl.

Alternatively, R^1 may be a monocyclic or bicyclic heterocyclic group having at least one ring nitrogen, oxygen or sulphur atom; the heterocyclic group may be aromatic or non-aromatic in character and examples include: 2-imidazolyl; 1,2,4-triazol-3-yl; 1,3,4-thiadiazol-2-yl; 2-pyridyl; 2-pyrimidyl; purin-6-yl; 2-benzothiazolyl; 2-benzoxazolyl; s-triazolo[4,3-a]pyridin-3-yl; and 2-thiazolyl.

The group A is a straight or branched-chain alkylene group, preferably a lower alkylene group having from 1 to 6 carbon atoms. Examples include: methylene, trimethylene, propylene, tetramethylene, 2-methyltetramethylene, pentamethylene and hexamethylene groups.

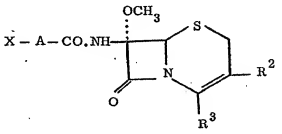
The group R^2 does not participate in the reaction of the process of the present invention and its nature is, therefore, not critical to the present invention.

However, preferred groups R^2 include: hydrogen atoms; lower alkyl groups, e.g. methyl; acyloxymethyl groups, e.g. acetoxymethyl; carbamoyloxymethyl groups; and thiomethyl groups which are S-substituted by aromatic monocyclic heterocyclic groups, containing at least one nitrogen, oxygen or sulphur ring atom, e.g. (1-methyl-1H-tetrazol-5-yl)thiomethyl or (1,3,4-thiadiazol-2-yl)thiomethyl.

In formula (II), the group R^3 is a carboxyl or esterified carboxyl group. When R^3 is an esterified carboxyl group, the nature of the ester is not particularly critical, provided that it does not destroy the cephem ring during or after the condensation reaction and provided that it can readily be split off from the cephem ring. Suitable esters include, for example: alkylsilyl esters, such as trimethylsilyl esters; lower alkyl esters, such as methyl, ethyl or t-butyl esters; benzyl esters; p-methoxybenzyl esters; benzhydryl esters; phenacyl esters; p-bromophenacyl esters; and 2,2,2-trichloroethyl esters. When R^3 is an esterified carboxyl group, the process of the invention preferably comprises the additional step of deesterifying the reaction product.

Where, as is preferred, the 7α-methoxycephalosporin to be produced is a compound of formula (II), defined above, the

preferred 7α-methoxycephalosporin starting material is a compound of formula (III):



(III)

(in which X, R^2 and R^3 are as defined above). In this compound, X is a halogen atom, preferably chlorine or bromine.

The thiol compound of formula (I) may be employed as such or may be, and preferably is, employed in the form of a metal salt, e.g. an alkali metal or alkaline earth metal salt, such as a sodium, potassium, lithium, calcium or barium salt. Of these, the sodium, potassium and lithium salts are preferred.

The process of the present invention can easily be carried out simply by contacting the 7α-methoxycephalosporin

derivative used as starting material, e.g. the compound of formula (III) with the thiol compound of formula (I), or with a metal salt thereof. In order that the reaction should proceed smoothly, we prefer that it should be carried out in the presence of a solvent, particularly if a metal salt of the thiol (I) is employed. Any solvent which does not participate in the reaction can be used in the process of the present invention, including water and many organic solvents. Examples of suitable organic solvents include dialkyl ketones, e.g. acetone or methyl ethyl ketone; halogenated alkanes, e.g. chloroform, methylene chloride or dichloroethane; lower alkanols, e.g. methanol or ethanol; and dimethylformamide. Mixtures of two or more solvents can also be employed. If the 7α-methoxycephalosporin derivative of formula (III) has a carboxyl group in the 4-position, we prefer to employ a mixture of water and an organic solvent as the reaction medium.

The thiol (I) or metal salt thereof will normally be used in an amount equimolar to the 7α-methoxycephalosporin derivative or in a slight excess; thus, for example, the molar ratio of thiol (I) to 7α-methoxycephalosporin starting material may be from 1 : 1 to 1,1 : 1.

Although the reaction will proceed at an approximately neutral pH, it proceeds more smoothly under weakly alkaline conditions. Accordingly, if the thiol (I) itself is employed, we prefer to add an alkali to the reaction mixture in order that the reaction should be carried out under weakly alkaline conditions. Suitable alkalis which may be used include: sodium hydroxide, potassium hydroxide, sodium carbonate, potassium carbonate, sodium bicarbonate and potassium bicarbonate.

The reaction temperature is not critical to the process of the invention and the reaction is, accordingly, normally effected at approximately ambient temperature, although the reaction will proceed at temperatures higher and lower than ambient. The time required for the reaction will vary depending upon the reaction temperature, the reagents and other conditions, but the reaction will normally be complete within from 2 to 30 hours; if X in the 7α-methoxycephalosporin starting material is a chlorine atom, the reaction will normally take a relatively long time, i.e. from 10 to 30 hours.

When the reaction is complete, the

desired compound, e.g. the compound of formula (II), may be recovered from the reaction mixture by conventional methods. For example, if a carboxyl group is present in the 4- position of the compound, the reaction mixture is first acidified and, if a solvent other than water is employed for the reaction, the solvent is removed by distillation, after which the reaction product is extracted with a suitable solvent (e.g. ethyl acetate) and the extract is washed with water and dried. The solvent is then removed by distillation, giving the desired product. If necessary, this may be further purified by conventional means, e.g. by chromatography.

If the compound produced by the process of the invention has an esterified carboxyl group at the 4- position, the reaction medium is, as stated above, preferably a mixture of water and a miscible organic solvent. This organic solvent is first removed from the reaction mixture by distillation, after which the residue is extracted with a water-immiscible solvent. The extract is then washed with water and the solvent removed by distillation to give the esterified compound. This compound may be further purified by conventional methods, e.g. chromatography, but, in general, we prefer that it should be

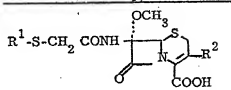
subjected directly to deesterification to give the desired compound. This deesterification can be carried out by any conventional method, depending upon the nature of the ester. Suitable deesterification processes include: alkaline hydrolysis, acid hydrolysis and reduction with hydrogen. After completion of the deesterification, the desired product is recovered from the reaction mixture in the manner described above.

Many of the 7α-methoxycephalosporin derivatives of formula (II) have valuable antibacterial activity against various pathogenic bacteria. Representative examples of these compounds and their antibacterial activities (expressed as minimium inhibitory concentrations) are shown in the following Table. Also shown, for purposes of comparison, are the minimum inhibitory concentrations against the same bacteria of two known compounds, 3-carbamoyloxymethyl-7α-methoxy-7β-phenylacetamido-3-cephem-4-carboxylic acid and Cephalothin.

As will be seen from the Table, the most preferred compounds of the invention are those in which: R^1 represents a cyanomethyl, 1-cyanoethyl, 2-hydroxyethyl, propargyl, azidomethyl or 3-isoxazolyl group; R^2 represents a (1-methyl-1H-tetrazol-5-yl)thiomethyl, carbamoyloxymethyl or acetoxymethyl group; and A represents a methylene group.

Table

Minimum Inhibitory Concentration (mcg/ml.)



Compound		S. aureus		E. coli		Sh.	Klebsiella		Prot.	Salm. ent.
R^1	R^2	209P	R	NIHJ	609 (R)	flexneri 2a	806	846	vulgaris	Gaertner
$NCCH_2$-		0.2	0.8	0.8	0.8	0.8	0.8	>200	1.5	0.2
NCCH- CH_3	"	0.4	0.8	1.5	3.1	1.5	1.5	400	0.8	0.4
$HOCH_2CH_2$-	"	0.8	1.5	1.5	1.5	3.1	1.5	400	6.2	1.5
$NCCH_2$-	$-CH_2OCONH_2$	0.8	3.1	1.5	1.5	3.1	3.1	>200	6.2	1.5
$NCCH_2$-	$-CH_2OCOCH_3$	0.2	0.8	1.5	1.5	3.1	1.5	>400	6.2	0.8

/Cont....

Table (cont)

Compound		S. aureus		E. coli		SH.	Klebsiella		Prot.	Salm. ent.
R^1	R^2	209P	R	NIHJ	609 (R)	flexneri 2a	806	846	vulgaris	Gaertner
$HC{\equiv}CCH_2$-		0.2	0.8	3.1	3.1	3.1	3.1	400	3.1	0.8
N_3CH_2-	"	0.4	0.4	3.1	3.1	1.5	3.1	>200	1.5	0.8
	"	0.2	0.8	3.1	3.1	3.1	6.2	>400	3.1	0.8
N_3CH_2-	$-CH_2OCONH_2$	0.2	1.5	3.1	6.2	3.1	3.1	>400	3.1	1.5
	$-CH_2OCOCH_3$	0.2	0.8	3.1	6.2	6.2	3.1	>400	3.1	1.5
3-carbamoyloxymethyl-7α-methoxy-7β-phenylacetamido-3-cephem-4-carboxylic acid		0.8	3.1	25	25	12.5	25	>400	25	12.5
Cephalothin		≤0.1	≤0.1	6.2	25	12.5	6.2	>200	6.2	6.2

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The compounds thus show excellent activity against both Gram- positive and Gram- negative bacteria, especially against Gram- negative bacteria.

The compounds of formula (III) which are used as starting materials in the process of the present invention are also novel compounds, but these can easily be prepared by known methods. Although they themselves have a certain antibacterial activity, they are more valuable as starting materials for the compounds of formula (II), which have much higher antibacterial activities.

The invention is further illustrated with reference to the following Examples. The preparation of certain novel starting materials is also illustrated in the following Preparations 1 to 4.

Example 1

3-Carbamoyloxymethyl-7α-methoxy-7β-(1,2,4-triazol-3-yl-thioacetamido)-3-cephem-4-carboxylic acid

12 mg of 3-mercapto-1,2,4-triazole were dissolved in 0.12 ml of a 1N aqueous solution of sodium hydroxide; to the resulting solution was added an aqueous solution of sodium hydrogen carbonate containing 42 mg of 7β-bromoacetamido-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid. The mixture was agitated at room temperature for 2 hours, and the pH was adjusted to about 2.5 by addition of 1N hydrochloric acid. The mixture was then extracted with ethyl acetate, and the organic phase was dried over anhydrous sodium sulphate. The solvent was then distilled off under reduced pressure, yielding 48 mg of a crystalline residue. This residue was chromatographed on a silica gel plate, employing a 50 : 50 by volume mixture of methanol and chloroform. The solvent was then distilled off, giving 33 mg of pure 3-carbamoyloxymethyl-7α-methoxy-7β-(1,2,4-triazol-3-yl-thioacetamido)-3-cephem-4-carboxylic acid in the form of a powder.

Nuclear magnetic resonance spectrum (CD_3CN + D_2O), δ ppm:

5.00 (singlet, H at 6-position)

3.90 (singlet, -S-CH_2-CO-)

3.37 (singlet, OCH_3 at 7-position).

Ultraviolet absorption spectrum (CH_3OH), λ_{max} mμ :

263.

Infrared absorption spectrum (KBr), ν cm^{-1} :

1770.

Thin layer chromatography (silica gel):

(a) Developing solvent (n-butanol/acetic acid/water, 5 : 4 : 1 by volume):

R_f value = 0.37.

(b) Developing solvent (methanol/chloroform, 1 : 1 by volume):

R_f value = 0.28.

When this procedure was repeated except that the 7β-bromoacetamido-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid was replaced by the corresponding 7β-chloroacetamido derivative, similar results were obtained.

Example 2

3-Carbamoyloxymethyl-7β-(imidazol-2-yl-thioacetamido)-7α-methoxy-3-cephem-4-carboxylic acid

20 mg of 2-mercaptoimidazole were dissolved in 0.2 ml of 1N sodium hydroxide, and a solution of 117 mg of 7β-bromoacetamido-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid in 0.2ml of 1N sodium hydroxide was added to the resulting solution. The mixture was allowed to stand at room temperature for 2 hours, after which the pH was adjusted to 2.5 by addition of 2N phosphoric acid. The mixture was then extracted with ethyl acetate and the aqueous phase was freeze-dried and extracted with methanol. The methanol-soluble portion was separated on a silica gel plate using a 50 : 50 by volume mixture of methanol and chloroform and extracted with methanol. The solvent was distilled from the extract, giving 80 mg of pure 3-carbamoyloxymethyl-7β-(imidazol-2-yl-thioacetamido)-7α-methoxy-3-cephem-4-carboxylic acid in the form of a powder.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

7.09 (singlet, H of imidazole)

5.02 (singlet, H at 6-position)

3.75 (singlet, $-S-CH_2-CO-$)

3.39 (singlet, OCH_3 at 7-position).

Ultraviolet absorption spectrum (phosphoric acid buffer, pH 6.86) λ_{max} mμ :

257.

Infrared absorption spectrum (KBr) ν cm^{-1} :

1765.

Thin layer chromatography (silica gel) :

(a) Developing solvent (n-butanol/acetic acid/water, 5 : 4 : 1 by volume) :

R_f value = 0.21.

(b) Developing solvent (methanol/chloroform, 1 : 1 by volume):

R_f = 0.31.

When this procedure was repeated except that the 7β-bromoacetamido-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid was replaced by the corresponding 7β-chloroacetamido derivative, similar results were obtained.

Example 3

3-Carbamoyloxymethyl-7α-methoxy-7β-(1,3,4-thiadiazol-2-yl-thioacetamido)-3-cephem-4-carboxylic acid

A solution of 117 mg of 7β-bromoacetamido-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid in 0.2 ml of 1N sodium hydroxide was added to a solution of

23.6 mg of 2-mercapto-1,3,4-thiadiazole in 0.2 ml of 1N sodium hydroxide, and the mixture was agitated at room temperature for 2 hours. The pH was then adjusted to 2.5 by addition of 2N phosphoric acid. The solution was then extracted 5 times, each time with 15 ml of ethyl acetate, and the combined ethyl acetate extracts were dried over sodium sulphate; the solvent was then distilled off, giving 69 mg of an amorphous crude product. This was separated on a silica gel plate using a 50 : 50 by volume mixture of methanol and chloroform and then extracted with methanol. The solvent was distilled off from the extract, giving 34 mg of 3-carbamoyloxymethyl-7α-methoxy-7β-(1,3,4-thiadiazol-2-yl-thioacetamido)-3-cephem-4-carboxylic acid in the form of a powder.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm :

9.30 (singlet, H of thiazole)

5.03 (singlet, H at 6-position)

4.16 (singlet, S-CH_2-CO-)

3.46 (singlet, OCH_3 at 7-position).

Ultraviolet absorption spectrum (phosphoric acid buffer, pH 6.86), λ_{max} mμ :

263.

Infrared absorption spectrum (KBr), ν cm^{-1} :

1760.

Thin layer chromatography (silica gel) :

(a) Developing solvent (n-butanol/acetic acid/water, 5 : 4 : 1 by volume):

R_f value = 0.49.

(b) Developing solvent (methanol/chloroform, 1 : 1 by volume):

R_f value = 0.29.

When this procedure was repeated except that the 7β-bromoacetamido-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid was replaced by the corresponding 7β-chloroacetamido derivative, similar results were obtained.

Examples of compounds prepared in the same manner as in the foregoing Examples and their properties are shown below.

Except where otherwise indicated, the physical properties were measured as follows:

Nuclear magnetic resonance spectrum (in CD_3CN + D_2O), δ ppm.

Ultraviolet absorption spectrum (in phosphoric acid buffer of pH 6.86), λ_{max} mμ.

Infrared absorption spectrum (in KBr), ν cm^{-1}.

D: Thin layer chromatography (TLC) on silica gel:

(a) R_f value obtained employing a 5 : 4 : 1 by volume mixture of n-butanol/acetic acid/water.

(b) R_f value obtained employing a 1 : 1 by volume mixture of methanol/chloroform.

3-Carbamoyloxymethyl-7α-methoxy-7β-(5-methyl-1,3,4-thiadiazol-2-yl-thioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.02 (singlet, H at 6-position)

4.05 (singlet, $S-CH_2CO-$)

3.43 (singlet, OCH_3 at 7-position)

2.64 (singlet, H of thiadiazole).

UV spectrum λ_{max} mμ :

263.

IR spectrum ν cm^{-1} :

1770.

TLC, R_f value:

(a)=0.45, (b)=0.38.

3-Carbamoyloxymethyl-7α-methoxy-7β-propargylthioacetamido-3-cephem-4-carboxylic acid

NMR spectrum [dimethylsulphoxide (DMSO)-d_6] , δ ppm:

5.15 (singlet, H at 6-position)

3.40 (multiplet, $-CH_2-S-CH_2$ and OCH_3 at 7-position).

UV spectrum, λ_{max} mμ:

242, 265.

IR spectrum ν cm^{-1} :

1780.

3-Carbamoyloxymethyl-7β-cyanomethylthioacetamido-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum (DMSO-d_6), δ ppm :

5.06 (singlet, H at 6-position)

3.58 (singlet, $NC-CH_2SCH_2$)

UV spectrum, λ_{max} mμ :

246, 265.

IR spectrum, ν cm^{-1}:

1720, 1775.

3-Carbamoyloxymethyl-7α-methoxy-7β-(5-methyl-1,2,4-triazol-3-yl-thioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum δ ppm :

5.02 (singlet, H at 6-position)

4.05 (singlet, SCH_2CO-)

3.43 (singlet, OCH_3 at 7-position)

2.64 (CH_3 of imidazole).

UV spectrum λ_{max} mμ :

263.

IR spectrum ν cm^{-1} :

1780.

TLC, R_f value:

(a)= 0.39, (b)=0.29.

3-Carbamoyloxymethyl-7β-(5-ethyl-1,2,4-triazol-3-yl-thioacetamido)-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum δ ppm:

5.13 (singlet, H at 6-position)

4.00 (singlet, $S-CH_2-CO$)

3.52 (singlet, OCH_3 at 7-position)

2.83 and 1.31 (quartet and triplet, CH_3CH_2 of triazole),

UV spectrum, λ_{max} mμ :

262.

IR spectrum, ν cm^{-1}:

1765.

TLC, R_f value:

(a)=0.46, (b)= 0.33.

3-Carbamoyloxymethyl-7α-methoxy-7β-(5-propyl-1,2,4-triazol-3-yl-thioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.07 (singlet, H at 6-position)

3.95 (singlet, $S-CH_2CO-$)

3.49 (singlet, OCH_3 at 7-position).

UV spectrum λ_{max} mμ :

263.

IR spectrum, ν cm^{-1}:

1765.

TLC, R_f value:

(a)=0.51, (b)=0.44.

3-Carbamoyloxymethyl-7α-methoxy-7β-(5-phenyl-1,2,4-triazol-3-yl-thioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum δ ppm:

4.94 (singlet, H at 6-position)

3.92 (singlet, $S-CH_2-CO-$)

3.38 (singlet, OCH_3 at 7-position)

7.3 - 8.0 (H of benzene).

UV spectrum λ_{max} mμ:

253, 235.

IR spectrum, ν cm^{-1}:

1765.

TLC, R_f value:

(a)=0.61, (b)0.53.

3-Carbamoyloxymethyl-7α-methoxy-7β-(4-phenyl-1,2,4-triazol-3-yl-thioacetamido)-3-cephem-4-carboxylic acid

acid

NMR spectrum, δ ppm:

4.99 (singlet, H at 6-position)

3.99 (singlet, SCH_2CO-)

3.41 (singlet, OCH_3 at 7-position).

UV spectrum λ_{max} mμ :

260.

IR spectrum, ν cm^{-1} :

1765.

TLC, R_f value:

(a)=0.43, (b)= 0.38.

3-Carbamoyloxymethyl-7α-methoxy-7β-(4-phenyl-5-methyl-1,2,4-triazol-3-yl-thioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.00 (singlet, H at 6-position)

3.92 (singlet, SCH_2CO-)

3.42 (singlet, OCH_3 at 7-position)

2.19 (singlet, CH_3 of triazole).

UV spectrum, λ_{max} mμ:

260.

IR spectrum, ν cm^{-1}:

1765.

TLC, R_f value:

(a)=0.41, (b)=0.41.

3-Carbamoyloxymethyl-7α-methoxy-7β-(2-pyridylthioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum δ ppm:

5.02 (singlet, H at 6-position)
3.89 (singlet, SCH_2CO-)
3.39 (singlet, OCH_3 at 7-position)
7.0 - 8.5 (H of pyridine).

UV spectrum, λ_{max} mμ:

266, 239.

IR spectrum, ν cm^{-1}:

1770.

TLC, R_f value:

(a)=0.57, (b)=0.43.

3-Carbamoyloxymethyl-7α-methoxy-7β-(4-methyl-2-pyrimidylthio-acetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.02 (singlet, H at 6-position)

3.93 (singlet, SCH_2CO-)

3.43 (singlet, OCH_3 at 7-position)

2.42 (singlet, CH_3 of pyrimidine).

UV spectrum, λ_{max} mμ:

242, 270.

IR spectrum, ν cm^{-1}:

1780.

TLC, R_f value:

(a)=0.51, (b)=0.39.

3-Carbamoyloxymethyl-7α-methoxy-7β-(2-pyrimidylthioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.04 (singlet, H at 6-position)

3.96 (singlet, SCH_2CO-)

3.46 (singlet, OCH_3 at 7-position)

7.22 and 8.58 (triplet and doublet, H of pyrimidine).

UV spectrum, λ_{max} mμ:

242, 265.

IR spectrum, ν cm^{-1}:

1760, 1700.

TLC, R_f value :

(a)=0.43, (b)=0.43.

3-Carbamoyloxymethyl-7α-methoxy-7β-(8-purinylthioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm :

4.99 (singlet, H at 6-position)

3.37 (singlet, OCH_3 at 7-position)

8.65 and 8.70 (H of purine).

UV spectrum, λ_{max} mμ:

287.

IR spectrum, $\nu_{cm^{-1}}$:

1770.

TLC, R_f value:

(a)=0.35, (b)=0.18.

3-Carbamoyloxymethyl-7α-methoxy-7β-(6-purinylthioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.01 (singlet, H at 6-position)

4.14 (singlet, SCH_2CO-)

3.43 (singlet, OCH_3 at 7-position)

8.31 and 8.63 (H of purine).

UV spectrum, λ_{max} mμ:

278.

IR spectrum, ν cm^{-1}:

1765.

TLC, R_f value:

(a)=0.40, (b) 0.20.

7β-(2-Benzimidazolylthioacetamido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

4.95 (singlet, H at 6-position)

4.16 (singlet, SCH_2CO-)

3.41 (singlet, OCH_3 at 7-position).

UV spectrum λ_{max} mμ:

281, 287.

IR spectrum, ν cm^{-1}:

1780, 1680.

TLC, R_f value:

(a)=0.48, (b)=0.51.

7β-(2-Benzoxazolylthioacetamido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.05 (singlet, H at 6-position)

4.14 (singlet, SCH_2CO-)

3.50 (singlet, OCH_3 at 7-position).

UV spectrum, λ_{max} mμ:

283, 276, 245.

IR spectrum ν cm^{-1}:

1780, 1720.

TLC, R_f value:

(a)=0.61, (b)=0.48.

7β-(2-Benzthiazolylthioacetamido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum (CD_3OD), δ ppm:

5.04 (singlet, H at 6-position)

3.45 (singlet, OCH_3 at 7-position).

UV spectrum, λ_{max} mμ :

297, 271.

IR spectrum, ν cm^{-1}:

1770.

TLC, R_f value:

(a)=0.58, (b)=0.48.

3-Carbamoyloxymethyl-7α-methoxy-7β-(s-triazolo[4,3-a] pyridin-3-yl-thioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

4.94 (singlet, H at 6-position)

3.95 (singlet, SCH_2CO-)

3.40 (singlet, OCH_3 at 7-position).

UV spectrum, λ_{max} mμ:

269.

IR spectrum, ν cm^{-1}:

1770, 1690.

TLC, R_f value:

(a)=0.29, (b)=0.39.

3-Carbamoyloxymethyl-7α-methoxy-7β-(2-naphthylthioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

4.92 (singlet, H at 6-position)
3.72 (singlet, SCH_2-CO-)
3.29 (singlet, OCH_3 at 7-position)
7.3 - 7.9 (H of naphthalene).

UV spectrum, λ_{max} mμ:

248, 270 (Sh.).

IR spectrum, ν cm^{-1}:

1765.

TLC, R_f value:

(a)=0.61, (b)=0.60.

["Sh" = "shoulder"]

3-Carbamoyloxymethyl-7α-methoxy-7β-(2-thiazolinylthioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.08 (singlet, H at 6-position)

3.92 (singlet, SCH_2CO-)

3.50 (singlet, OCH_3 at 7-position).

UV spectrum, λ_{max} mμ:

258, 239.

IR spectrum ν cm^{-1}:

1770, 1690.

TLC, R_f value:

(a)=0.49, (b)=0.49.

3-Carbamoyloxymethyl-7β-cyclohexylthioacetamido-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.08 (singlet, H at 6-position)

3.81 (singlet, SCH_2CO-)

3.48 (singlet, OCH_3 at 7-position)

1.0 - 2.0 (CH_2 of cyclohexane).

UV spectrum, λ_{max} mμ:

266.

TLC, R_f value:

(a)=0.56, (b)=0.50.

3-Carbamoyloxymethyl-7α-methoxy-7β-(n-propylthioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum (CD_3OD), δ ppm:

5.04 (singlet, H at 6-position)

3.45 (singlet, OCH_3 at 7-position).

UV spectrum, λ_{max} mμ:

265.

IR spectrum, νcm^{-1}:

1770.

TLC, R_f value:

(a)=0.55, (b)=0.55.

3-Carbamoyloxymethyl-7α-methoxy-7β-(n-pentylthioacetamido)-3-cephem-4-carboxylic acid

NMR spectrum (CD_3OD), δ ppm:

5.04 (singlet, H at 6-position)

3.46 (singlet, OCH_3 at 7-position).

UV spectrum, $\lambda_{max.}$ mμ:

265.

IR spectrum, $\nu_{cm^{-1}}$:

1770.

TLC, R_f value:

(a)=0.60, (b)=0.54.

Example 4

7β-(2-Butylthioacetamido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid

45 mg of sec-butanethiol were dissolved with 0.5 ml of a 1N aqueous solution of sodium hydroxide in 50% aqueous methanol, and 0.5 ml of benzhydryl 7β-bromoacetamido-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylate was added to the resulting solution. The mixture was agitated for 2 hours, after which methanol was distilled off under reduced pressure. The aqueous phase was extracted three times with ethyl acetate and the extracts were dried over sodium sulphate. The solvent was then distilled off, giving crude benzhydryl 7β-

(2-butylthioacetamido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylate. This crude product was dissolved in 1.0 ml of anisole, and 1.5 ml of trifluoroacetic acid were added to the resulting solution. The mixture was allowed to stand at room temperature for 6 minutes and then evaporated to dryness under reduced pressure. The solid residue was dissolved in 20 ml of ethyl acetate and 20 ml of 0.2M phosphoric acid buffer (pH 7.5) and the mixture was agitated and then allowed to stand to allow phase separation. The aqueous phase was washed once again with ethyl acetate and its pH was then adjusted to 2.5. The aqueous layer was then extracted 4 times with 20 ml of ethyl acetate, and the combined extracts were dried over sodium sulphate and evaporated to dryness under reduced pressure giving an amorphous crude product. This product was separated and purified on a silica gel plate employing a 50 : 50 mixture of methanol and chloroform. 115 mg of the desired product were obtained in the form of a powder.

Nuclear magnetic resonance spectrum (CD_3OD), δ ppm:

5.08 (singlet, H at 6-position)

3.58 (singlet, OCH_3 at 7-position).

Ultraviolet absorption spectrum (CH_3OH) λ_{max} mμ:

267 (ε = 9140).

Infrared absorption spectrum (KBr), ν cm^{-1}:

1795, 1720, 1680.

Thin layer chromatography (silica gel):

(a) Developing solvent (n-butanol/acetic acid/water, 5 : 4 : 1 by volume):

R_f value = 0.59.

(b) Developing solvent (chloroform/methanol, 1 : 1 by volume):

R_f value = 0.60.

Example 5

3-Acetoxymethyl-7α-methoxy-7β-(1,3,4-thiadiazol-2-yl-thioacetamido)-3-cephem-4-carboxylic acid

33 mg of 2-mercapto-1,3,4-thiadiazole were dissolved in 0.24 ml of 1N aqueous sodium hydroxide, and a solution of 0.27 mmole (100 mg) of 3-acetoxymethyl-7β-chloroacetamido-7α-methoxy-3-cephem-4-carboxylic acid in 0.5N sodium bicarbonate was added to the resulting solution. The mixture was agitated at room temperature for 3 hours and the pH was then adjusted to 2.5 by addition of 0.1N phosphoric acid. The mixture was then extracted 3 times with ethyl acetate and the combined ethyl acetate extracts were dried over sodium sulphate. The solvent

was distilled off from the dried ethyl acetate solution, giving 113 mg of an amorphous crude product. This was separated on a silica gel plate employing chloroform containing 40% methanol as a solvent and was then extracted with methanol. The solvent was distilled off from the extract, giving 69 mg of the desired product.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

9.23 (singlet, H of thiadiazole)

5.00 (singlet, H at 6-position)

4.73 (doublet, CH_2 at 3-position)

4.11 (singlet, $S-CH_2-CO-$)

3.42 (singlet, OCH_3 at 7-position)

1.98 (singlet, $O-CO-CH_3$).

Ultraviolet absorption spectrum (phosphoric acid buffer of pH 6.86), λ_{max} mμ:

263 mμ (ϵ =8789).

Infrared absorption spectrum (KBr), ν cm^{-1}:

1760.

Thin layer chromatography (silica gel):

Developing solvent (methanol/chloroform, 1:1 by volume):

R_f value = 0.45.

Examples of other compounds prepared in the same manner as in the foregoing Example, together with

their properties (determined as described in Example 3) are shown below:

3-Acetoxymethyl-7β-cyanomethylthioacetamido-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum (CD_3CN), δ ppm:

5.06 (singlet, H at 6-position)

4.76 - 5.06 (quartet, $-CH_2OCO-$ at 3-position)

3.60 (singlet, $NCCH_2S$ or SCH_2CO)

3.52 (singlet, OCH_3 at 7-position)

3.42 (singlet, $NCCH_2S$ or SCH_2CO)

3.32 - 3.55 (quartet, H_2 at 2-position)

2.02 (singlet, $OCOCH_3$).

UV spectrum λ_{max} mμ:

247 (ε = 8000)

267 (ε =8400).

IR spectrum, ν cm^{-1}:

1775.

3-Acetoxymethyl-7α-methoxy-7β-propargylthioacetamido-3-cephem-4-carboxylic acid

NMR spectrum (DMSO-d_6), δ ppm:

5.05 (singlet, H at 6-position)

4.9 - 4.6 (quartet, $-CH_2OCO-$ at 3-position)

3.36 (singlet, OCH_3 at 7-position)

3.2 - 3.5 (multiplet, 2-position, H_2, $-CH_2$ and $-S-CH_2-$)

3.05 (triplet, $HC\equiv C$)

1.98 (singlet, $OCOCH_3$).

UV spectrum, λ_{max} mμ:

245 (ε =7800)

268 (ε =8200).

IR spectrum, ν cm^{-1}:

1775.

When 83 mg of the carboxylic acid thus obtained were added to 8 ml of water, 17 mg of sodium bicarbonate were gradually added thereto and the resulting homogeneous solution was freeze-dried, a sodium salt of the carboxylic acid was obtained in the form of an amorphous powder.

3-Acetoxymethyl-7β-azidomethylthioacetamido-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum (DMSO-d_6), δ ppm:

5.14 (singlet, H at 6-position)

4.69 - 4.98 (quartet, $-CH_2-OCO-$ at 3-position)

4.51 (singlet, N_3CH_2S-)

3.40 (singlet, 7-position, OCH_3, $S\text{-}CH_2\text{-}CO$)

3.3 - 3.6 (quartet, H_2 at 2-position)

2.00 (singlet, $OCO\text{-}CH_3$).

UV spectrum, λ_{max} mμ :

247 (ε = 7800)

269 (ε = 8000).

IR spectrum, ν cm^{-1} :

1775.

3-Acetoxymethyl-7β-(imidazol-2-yl-thioacetamido)-7α-methoxy-3-cephem-4-carboxylic acid

NMR spectrum δ ppm:

7.11 (singlet, H at 4- and 5-position of imidazole)

5.02 (singlet, H at 6-position)

4.78 (broad singlet, H_2 at 3-position)

3.78 (singlet, $-S\text{-}CH_2CO-$)

3.38 (singlet, OCH_3 at 7-position)

2.02 (singlet, $-O\text{-}COCH_3$).

UV spectrum, λ_{max} mμ :

265.

IR spectrum, ν cm^{-1} :

1760.

TLC, R_f value:

(b) =0.46.

Example 6

7α-Methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-7β-(1,3,4-thiadiazol-2-yl-thioacetamido)-3-cephem-4-carboxylic acid

33 mg of 2-mercapto-1,3,4-thiadiazole were dissolved in 0.24 ml of 1N sodium hydroxide and a solution of 117 mg (0.27 mmole) of 7β-chloroacetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid in 0.5N sodium hydrogen carbonate was added to the resulting solution. The mixture was agitated at room temperature for 3 hours, and the pH was then adjusted to 2.5 by addition of 0.1N phosphoric acid. The mixture was extracted 3 times with ethyl acetate, and the combined extracts were dried over sodium sulphate. The solvent was then distilled off, giving 125 mg of an amorphous product. This was separated on a silica gel plate employing a 50 : 50 mixture of methanol and chloroform and extracted with methanol. The solvent was distilled off from the extract, giving 70 mg of the desired product in the form of an amorphous powder.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

9.28 (singlet, H at 5-position of thiadiazole).

Ultraviolet absorption spectrum (phosphoric acid buffer of pH 6.86) λ_{max} mμ:

266 (ε = 9270).

Infrared absorption spectrum (KBr), ν cm^{-1}:

1760.

Thin layer chromatography (silica gel):

Developing solvent (chloroform/methanol, 1:1 by volume)

R_f value = 0.38.

Examples of compounds prepared in the same manner as in the foregoing Example and their properties (measured as described in Example 3) are shown below:

7β-(Imidazol-2-ylthioacetamido)-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid

NMR spectrum δ ppm:

7.03 (singlet, H at 4- and 5-position of imidazole)

4.95 (singlet, H at 6-position)

3.84 (singlet, CH_3 at 1-position of tetrazole ring)

3.36 (singlet, OCH_3 at 7-position).

UV spectrum, λ_{max} mμ:

265 (ε = 7200).

IR spectrum, ν cm^{-1}:

1760.

TLC, R_f value:

(b) = 0.36.

7β-Cyanomethylthioacetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid

NMR spectrum, δ ppm:

5.10 (singlet, H at 6-position)

4.3 - 4.6 (quartet, CH_2-S at 3-position)

3.98 (singlet, CH_3 at 1-position of tetrazole ring)

3.70 (singlet, -$NCCH_2S$ or -SCH_2CO)

3.5 - 3.7 (quartet, H_2 at 2-position)

3.60 (singlet, -$NCCH_2S$ or -SCH_2CO)

3.50 (singlet, OCH_3 at 7-position).

UV spectrum, λ_{max} mμ:

274 (ε = 9000).

7α-Methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-7β-propargylthioacetamido-3-cephem-4-carboxylic acid

NMR spectrum (DMSO-d_6), δ ppm:

5.05 (singlet, H at 6-position)

4.2 - 4.3 (quartet, CH_2-S at 3-position)

3.90 (singlet, CH_3 at 1-position of tetrazole ring)

about 3.5 (multiplet, OCH_3 at 7-position, H_2,

-CH_2-S-CH_2CO at 2-position)

3.20 (triplet, HC≡C-).

Example 7

7α-Methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-7β-(1,3,4-thiadiazol-2-yl-thioacetamido)-3-cephem-4-carboxylic acid

To a solution of 596 mg of 2-[7β-bromoacetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carbonyl] -s-triazolo[4,3-a] pyrid-3-one in 30 ml of acetone was added a solution of 86 mg of 2-mercapto-1,3,4-thiadiazole and 84 mg of sodium hydrogen carbonate in 8 ml of water; the resulting mixture was stirred at room temperature for 1 hour. The solvent was then distilled off under reduced pressure and 50 ml of ethyl acetate were added to the residue, which was then washed with water and dried over anhydrous magnesium sulphate. The solvent was distilled off, giving crude 2-[7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-7β-(1,3,4-thiadiazol-2-yl-thioacetamido)-3-cephem-4-carbonyl] -s- triazolo[4,3-a] pyrid-3-one as a powder.

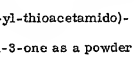
Nuclear magnetic resonance spectrum ($CDCl_3$), δ ppm:

3.43 (singlet, O-CH_3 at 7-position)

3.90 (singlet, N-CH_3 at 3-position of tetrazole)

4.25 (singlet, -S-CH_2-CO at 7-position)

5.24 (singlet, 6-position, )

6.3 - 7.9 (multiplet, 4-position -CO-N )

9.08 (7-position, thiadiazole).

Ultraviolet absorption spectrum (THF), λ_{max} nm:

227, 261, 359.

Infrared absorption spectrum (Nujol-trade mark), $\nu_{cm^{-1}}$:

1770, 1700, 1650.

Thin layer chromatography (silica gel):

(a) Developing solvent (chloroform containing 10% methanol):

R_f value = 0.56.

(b) Developing solvent (n-butanol/acetic acid/water, 4 : 1 : 1 by volume):

R_f value = 0.47.

To a solution of the compound obtained above in a mixture of 15 ml of tetrahydrofuran and 7.5 ml of water were added 600 mg of copper acetate monohydrate. The resulting mixture was stirred at room temperature for 5 hours, after which 50 ml of ethyl acetate and 7.5 ml of a 0.5M citric acid solution were added thereto. Insolubles were filtered off and the organic phase was washed three times with 50 ml of an aqueous solution of sodium chloride and then dried over anhydrous magnesium sulphate. The solvent was distilled off under reduced pressure to give a crude amorphous product,

which was purified by thin layer chromatography, using as solvent a mixture of n-butanol/acetic acid/water (4 : 1 : 1 by volume), to give the desired product.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

3.46 (singlet, O-CH_3 at 7-position)

3.94 (singlet, 3-position, tetrazole N-CH_3)

5.02 (singlet, 6-position, )

9.28 (singlet, 7-position, ).

Infrared absorption spectrum (KBr) ν cm^{-1}:

1760.

Ultraviolet absorption spectrum (in phosphoric acid buffer of pH 6.86), λ_{max} nm:

266.

Thin layer chromatography (silica gel):

Developing solvent (chloroform/methanol, 1 : 1 by volume):

R_f value = 0.38.

Example 8

7β-(2-Carboxyphenylthioacetamido)-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid

To a solution of 596 mg of 2-[7β-bromoacetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carbonyl] -s-triazolo[4,3-a] pyrid-3-one in 30 ml of acetone was added a solution of 152 mg of thiosalicylic acid and 173 mg of sodium bicarbonate in 8 ml of water. The resulting mixture was stirred at room temperature for 30 minutes. The solvent was then distilled off under reduced pressure and 50 ml of ethyl acetate were added to the residue. The resulting solution was then washed with water and dried over anhydrous magnesium sulphate. The solvent was then distilled off under reduced pressure, giving crude 2-[7β-(2-carboxyphenylthioacetamido)-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carbonyl] -s-triazolo-[4,3-a] pyrid-3-one as a powdery residue.

This product was dissolved in a mixture of 15 ml of tetrahydrofuran and 7.5 ml of water and then 600 mg of copper acetate monohydrate were added thereto. The resulting mixture was stirred at room temperature for 5 hours, after which 50 ml of ethyl acetate and 7.5 ml of a 0.5M citric acid solution were added thereto. Insolubles were filtered off and the organic phase was washed three times with an aqueous solution of sodium chloride and then dried over anhydrous magnesium sulphate.

After the solvent had been distilled off under reduced pressure, the residue was purified by thin layer chromatography on silica gel, using as solvent a mixture of n-butanol/acetic acid/water (4 : 1 : 1 by volume), to give the desired product.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

7.2 - 7.6 (multiplet, proton in phenyl moiety)

5.13 (singlet, 6-position, )

4.07 (singlet, 3-position tetrazole $>N-CH_3$)

3.47 (singlet, 7-position, $-O-CH_3$).

Infrared absorption spectrum (KBr) ν cm^{-1}:

1775.

Ultraviolet absorption spectrum (phosphoric acid buffer, pH 6.86), λ_{max} nm:

253.

Thin layer chromatography (silica gel):

(a) Developing solvent (n-butanol/acetic acid/water, 4 : 1 : 1 by volume):

R_f value = 0.36.

(b) Developing solvent (chloroform/methanol/water, 6 : 4 : 1 by volume):

R_f value = 0.20.

Example 9

7β-(2-Hydroxyethylthioacetamido)-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid

434 mg of 7β-chloroacetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid were dissolved in 20 ml of water containing 84 mg of sodium bicarbonate and 156 mg of thioglycol were added thereto. The resulting mixture was then stirred at room temperature for 3 hours, while maintaining the pH at 8.0-8.5 by addition of a 1N sodium hydroxide solution. The pH was then adjusted to 2.0 - 2.5 by addition of 1N hydrochloric acid and the mixture was freeze-dried. The residue was extracted with methanol and the solvent was distilled off. The resulting residue was then purified by preparative silica gel chromatography using as developing solvent a mixture of n-butanol/acetic acid/water (4 : 1 : 1 by volume), to give 360 mg of the desired product as a powder.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

5.20 (singlet, 6-position, )

4.15 - 4.25 (quartet, 3-position, )

3.90 (singlet, 3-position, tetrazole )

3.44 (singlet, 7-position, $-O-CH_3$)

3.30 (singlet, 7-position, $-S-CH_2-\overset{O}{\overset{\|}{C}}-$)

3.68 and 2.74 (triplet, 7-position, $HO-CH_2CH_2-S-$).

Infrared absorption spectrum (KBr), ν cm^{-1}:

1740, 1675.

Ultraviolet absorption spectrum (phosphoric acid buffer, pH 6.86), λ_{max} mμ:

270 (ε = 9450).

Preparation 1

7β-Bromoacetamido-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid

Into a beaker were charged 808 mg of bromoacetyl bromide and 1.030 g of bis-(trimethylsilyl)trifluoroacetamide and the resulting mixture was allowed to stand at room temperature for 20 minutes. 5 ml of methylene chloride were then added to the mixture, followed by a solution of 879 mg of dibenzhydryl 7β-(D-5-t-butoxycarbonylamino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylate in 5 ml of methylene chloride. The beaker was washed with 10 ml of methylene chloride and the washings were combined with the mixture. The mixture was allowed to stand at room temperature under moist conditions for 2 hours. 1 g of sodium bicarbonate was then added to the reaction mixture,

whilst cooling with ice/sodium chloride, and then 20 ml of a 5% aqueous solution of sodium bicarbonate was added to the reaction mixture, which was agitated for 30 minutes. The methylene chloride phase was combined with the washings obtained by washing the water phase with 10 ml of methylene chloride, and the mixture was washed twice with 20 ml of a 20% aqueous solution of sodium chloride. The organic phase was dried over sodium sulphate and the solvent was distilled off under reduced pressure, giving 1.194 g of crude dibenzhydryl 7β-[(D-5-t-butoxycarbonylamino-5-carboxyvaleryl)-bromo-acetylamino] -3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylate in the form of a yellow amorphous product.

This yellow product was dissolved in 1 ml of anisole and 2 ml of trifluoroacetic acid, and the resulting solution was allowed to stand at room temperature for 5 minutes, after which the solvent was distilled off under reduced pressure. The resulting yellow viscous paste was dissolved in 20 ml of ethyl acetate and 20 ml of a 0.2M phosphoric acid buffer (pH 7.5), and the solution was then transferred into a separating funnel and well shaken. After phase separation, the pH of the aqueous phase was adjusted to 2.5 with 1N hydrochloric

acid and the aqueous phase was extracted 5 times with 20 ml of ethyl acetate. The extracts were dried over sodium sulphate, after which the solvent was distilled off under reduced pressure. 300 mg of the desired compound were obtained in the form of a crude amorphous product.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

5.12 (singlet, H at 6-position)

3.95 (singlet, $BrCH_2CO$)

3.53 (singlet, OCH_3 at 7-position).

Ultraviolet absorption spectrum (CH_3OH), λ_{max}, mμ:

263.

Infrared absorption spectrum (KBr), ν cm^{-1}:

1780, 1700.

Thin layer chromatography (silica gel):

(a) Developing solvent (n-butanol/acetic acid/ water, 5 : 4 : 1 by volume):

R_f value = 0.53.

(b) Developing solvent (methanol/chloroform, 1:1 by volume):

R_f value = 0.44.

Preparation 2

3-Carbamoyloxymethyl-7β-chloroacetamido-7α-methoxy-3-cephem-4-carboxylic acid

The procedure described in Preparation 1 was repeated except that chloroacetyl chloride was employed instead of the bromoacetyl bromide. Separation and purification took place as described in Preparation 1, giving the desired compound in the form of a crude amorphous product.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

5.1 (singlet, H at 6-position)

3.48 (singlet, OCH_3 at 7-position)

4.11 (singlet, $ClCH_2CO$).

Ultraviolet absorption spectrum (phosphoric acid buffer, pH 6.86), λ_{max}, mμ:

264.

Infrared absorption spectrum (KBr), ν cm^{-1}:

1780, 1700.

Thin layer chromatography (silica gel):

Developing solvent (methanol/chloroform, 1 :1 by volume):

R_f value = 0.35.

Preparation 3

3-Acetoxymethyl-7β-chloroacetamido-7α-methoxy-3-cephem-4-carboxylic acid

438.5 mg of benzhydryl 3-acetoxymethyl-7β-chloroacetamido-7α-methoxy-3-cephem-4-carboxylate were

dissolved in 0.4 ml of anisole, and 0.8 ml of trifluoroacetic acid was added to the resulting solution. The mixture was agitated at room temperature for 5 minutes. The reaction mixture was then promptly evaporated to dryness and the residue was washed with n-hexane and dissolved in 5 ml of 0.25M phosphoric acid buffer (pH 7.5). The resulting solution was neutralized with a 5% aqueous solution of sodium bicarbonate and washed with ethyl acetate. The pH of the aqueous phase was adjusted to 2.0 with 60% phosphoric acid and the oily substance which precipitated was extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulphate, after which the solvent was distilled off, giving 276 mg of the desired compound in the form of an amorphous powder.

Nuclear magnetic resonance spectrum ($CD_3CN + D_2O$), δ ppm:

5.08 (singlet, H at 6-position)

4.08 (singlet, $ClCH_2CO$)

3.55 (singlet, OCH_3 at 7-position).

Thin layer chromatography (silica gel):

Developing solvent (chloroform/methanol, 9 : 1 by volume):

R_f value = 0.41.

Preparation 4

7β-Chloroacetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid

27.6 g of disodium 7β-(D-5-amino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylate were dissolved in 1090 ml of a 5% aqueous solution of dipotassium phosphate, and 715 ml of acetone were added to the resulting solution. 8.1 g of 4-dimethylaminopyridine were then added to the solution, whose pH was adjusted to 9.5 by addition of 2.5N aqueous sodium hydroxide, following which 34.5 ml of t-butoxycarbonyl azide were added and the mixture was agitated at room temperature for 4 hours while maintaining the pH at 9.0 to 9.5. The reaction mixture was allowed to stand at 4°C. overnight, at the end of which time 1000 ml of ethyl acetate were added thereto, and the mixture was well shaken. The aqueous phase was collected and another 1000 ml of ethyl acetate were added thereto, after which the pH of the aqueous phase was adjusted to 2.5 by addition of concentrated hydrochloric acid, while maintaining the temperature at 0° to 2°C. The organic phase was separated and the aqueous phase was further extracted twice with 1200 ml of ethyl acetate. The organic phases were combined and washed with a saturated aqueous solution of sodium chloride until the pH of the washings reached 4 - 5, when they were dried

with anhydrous sodium sulphate. On distilling off the solvent from the solution, there were obtained 22.1 g of 7β-(D-5-t-butoxycarbonylamino-5-carboxyvaleramido)-3-carbamoyloxymethyl-7α-methoxy-3-cephem-4-carboxylic acid.

This compound was added to a phosphoric acid buffer of pH 7.0 containing 10 g of 5-mercapto-1-methyl-1H-tetrazole, and the mixture was agitated at 95°C. for 30 minutes, after which the pH was adjusted to 2.5 by addition of hydrochloric acid, with ice-cooling. The reaction mixture was then extracted with ethyl acetate, and the extracts were washed with a saturated aqueous solution of sodium chloride until the pH of the washings reached 4 - 5, after which the extracts were dried over anhydrous sodium sulphate. A solution of 20 g of diphenyldiazomethane in ether was added to the dried extracts and the mixture was agitated for 2 hours, after which it was washed first with a 20% aqueous solution of sodium chloride and then with a 5% aqueous solution of sodium bicarbonate. The washed mixture was dried over anhydrous sodium sulphate and the solvents were distilled off; the residue was then dissolved in chloroform, adsorbed on a column packed with silica gel and eluted with chloroform containing 1% v/v of methanol. Distilling off the solvent from the eluent gave dibenzhydryl 7β-(D-5-t-butoxycarbonylamino-5-

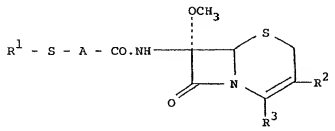
carboxyvaleramido)-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl) thiomethyl-3-cephem-4-carboxylate.

5 mmole of this compound were dissolved in 50 ml of chloroform, and the solution was added to a mixture of 2.26 g of chloroacetyl chloride and 2.26 g of bis-(trimethylsilyl)trifluoroacetamide, which had previously been allowed to stand at room temperature for 30 minutes, and the resulting mixture was allowed to stand at 40°C for 100 hours. The reaction mixture was then poured into a 5% aqueous solution of sodium bicarbonate and the mixture was agitated for 30 minutes. The organic phase was collected and washed with a 20% aqueous solution of sodium chloride, and then dried over anhydrous sodium sulphate. The solvent was distilled off from the washed solution and the residue was dissolved in 5 ml of anisole and 10 ml of trifluoroacetic acid. This solution was shaken at room temperature for 5 minutes and then evaporated to dryness under reduced pressure. The residue was dissolved in 1M phosphoric acid buffer (pH 7.5) and extracted with ethyl acetate. The aqueous phase was collected and its pH adjusted to 2.5 by addition of 1N hydrochloric acid; it was then extracted with ethyl acetate. The extract was dried over anhydrous sodium sulphate, and the solvent was distilled

off, giving 7β-chloroacetamido-7α-methoxy-3-(1-methyl-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid in the form of an amorphous powder.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A process for preparing a 7α-methoxy-cephalosporin derivative of formula



(II)

where R^1 is a hydrogen atom; a straight or branched- chain hydrocarbon group having from 1 to 6 carbon atoms which is unsubstituted or which has one or more hydroxy, azido, cyano, nitro, acylamino, alkoxycarbonylamino, alkoxy, phenyl, cyclohexadienyl, cyclohexyl or alkoxycarbonyl substituents; a phenyl or naphthyl group which is unsubstituted or which has one or more halogen, alkyl, alkoxy, cyano, nitro, acylamino or alkoxycarbonylamino substituents; a saturated or unsaturated monocyclic hydrocarbon group having a 5- or 6- membered ring; an alkanoyl group having from 2 to 18 carbon atoms, which is unsubstituted or which has one or more cyano, nitro, acylamino, alkoxycarbonylamino or alkoxycarbonyl substituents; a benzoyl group which is unsubstituted or which has one or more halogen, alkyl, alkoxy, cyano, nitro, acylamino or alkoxycarbonylamino substituents; a 2-imidazolyl group; a 1,2,4-triazol-3-yl group; a 1,3,4-thiadiazol-2-yl group; a 2-pyridyl group; a 2-pyrimidyl group; a purin-6-yl group; a 2-benzothiazolyl group; a 2-benzoxazolyl group; an s-triazolo [4,3-a] pyridin-3-yl group; or a 2-thiazolyl group, R^2 is an acyloxymethyl group, a carbamoyloxymethyl group or a thiomethyl group which is S- substituted by an aromatic monocyclic heterocyclic group having at least one ring nitrogen, oxygen or sulphur atom; R^3 is a carboxyl group or an esterified carboxyl group and A is methe-

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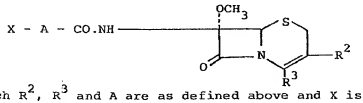
lene wherein 7α-methoxycephalosporin of formula (III):



(III)

in which R^2, R^3 and A are as defined above and X is a halogen atom reacted with a thiol compound of formula (I):

$$R^1 - SH \qquad (I)$$

or with a metal salt thereof wherein R^1 is as above.

2. A process as claimed in Claim 1, wherein an alkali metal or alkaline earth metal salt of said thiol compound (I) is employed.

3. A process as claimed in Claim 1, wherein a sodium potassium or lithium salt of said thiol compound (I) is employed.

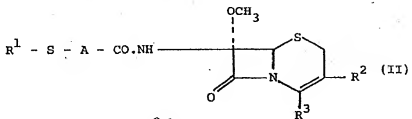
4. A process as claimed in Claim 1, wherein when R^3 is a carboxyl group the reaction is effected in the presence of a mixture of water and a water-miscible organic solvent.

5. A process as claimed in Claim 1, wherein when R^3 is a carboxyl group the reaction is effected in a mixture of water and methanol or ethanol.

6. A process as claimed in Claim 1, wherein said thiol compound (I) itself is employed and the reaction is effected in the presence of an alkali.

7. A process as claimed in Claim 6, wherein said alkali is sodium hydroxide, potassium hydroxide, sodium carbonate, potassium carbonate, sodium bicarbonate or potassium bicarbonate.

8. A 7α-methoxycephalosporin derivative of formula (II):



(II)

in which R^1 is a hydrogen atom; a straight or branched- chain hydrocarbon group having from 1 to 6 carbon atoms which is unsubstituted or which has one or more hydroxy, azido, cyano, nitro, acylamino, alkoxycarbonylamino, alkoxy, phenyl, cyclohexadienyl, cyclohexyl or alkoxycarbonyl substituents: a phenyl or naphthyl group which is unsubstituted or which has one or more halogen, alkyl, alkoxy, cyano, nitro, acylamino or alkoxycarbonylamino substituents; a saturated or unsaturated monocyclic hydrocarbon group having a 5- or 6- membered ring; an alkanoyl group having from 2 to 18 carbon atoms, which is unsubstituted or which has one or more cyano, nitro, acylamino, alkoxycarbonyl amino or alkoxycarbonyl substituents; a benzoyl group which is unsubstituted or which has one or more halogen, alkyl, alkoxy, cyano, nitro, acylamino or alkoxycarbonylamino substituents; a 2-imidazolyl group; a 1,2,4-triazol-3-yl group;, a 1,3,4-thiadiazol-2-yl group; a 2-pyridyl group; a 2-pyrimidyl group; a purin-6-yl group; a 2-benzothiazolyl group; a 2-benzoxazolyl group an s-triazolo [4,3,-a] pyridin-3-yl group; or a 2-thiazolyl group, R^2 is an acyloxymethyl group, a carbamoyloxymethyl group or a thiomethyl group which is S-substituted by an aromatic monocyclic heterocyclic group having at least one ring nitrogen, oxygen or sulphur atom, R^3 is a carboxyl group or esterified carboxyl group; and A is a methylene group whenever prepared or produced by the process as claimed in Claim 1, 2 or 3 or an obvious chemical equivalent thereof.

9. A process as claimed in Claim 1, wherein the reactants R^2 is a (1-methyl-1H-tetrazol-5-yl)-thiomethyl, carbamoyloxymethyl or acetoxymethyl group.

10. A derivative of formula II given in Claim 1, in which R^1, R^3 and A are as in Claim 1 and R^2 is as in Claim 9, whenever prepared or produced by the process as claimed in Claim 9, or an obvious chemical equivalent thereof.

11. A process as claimed in claim 1 wherein in the reactants R^1 is a cyanomethyl, 1-cyanoethyl, 2-hydroxyethyl, propargyl, azidomethyl or 3-isoxazolyl group.

12. A derivative of formula II given in claim 1 in which R^2, R^3 and A are as in claim 1 and R^1 is as in claim 11 whenever prepared or produced by the process as claimed in claim 11 or an obvious chemical equivalent thereof.

13. A process as claimed in claim 1 wherein in the reactants R^1 is a cyanomethyl, 1-cyanoethyl, 2-hydroxyethyl, propargyl, azidomethyl or 3-isoxazolyl group and R^2 is a (1-methyl-1H-tetrazol-5-yl) thiomethyl group, a carbamoyloxymethyl group or an acetoxymethyl group.

14. A derivative of formula II given in claim 1 in which A and R^3 are as in claim 1 and R^1 and R^2 are as in claim 13 whenever prepared or produced by the process as claimed in claim 13 or an obvious chemical equivalent thereof.

15. A process as claimed in claim 13 in which in the reactants R^3 is a carboxyl group or a lower alkyl silyl ester; lower alkyl ester; a benzyl ester; a p-methoxy-benzyl ester; a benzylhydryl ester; a phenacyl ester; a p-bromo-phenacyl ester or a 2,2,2-trichloroethyl ester group.

16. A derivative of formula II given in claim 1 in which R^1 and R^2 are as in claim 13, A is methylene and R^3 is as in claim 15 whenever prepared or produced by the process as claimed in claim 15 or an obvious chemical equivalent thereof.

17. A process as claimed in claim 13 in which in the reactants R^3 is a carboxyl group.

18. A derivative of formula II given in claim 1 in which R^1 and R^2 are as in claim 13 and A is methylene and R^3 is as in claim 17 whenever prepared or produced by the process as claimed in claim 17 or an obvious chemical equivalent thereof.

19. A process as claimed in claim 1 in which in the reac-

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tants R^3 is a carboxyl, R^1 is cyanomethyl, 1-cyanoethyl, 2-hydroxyethyl, propargyl, azidomethyl, or 3-isoxazolyl, and R^2 is a (1-methyl-1H-tetrazol-5-yl)-thiomethyl, a carbamoyloxymethyl or an acetoxy methyl group.

20. A derivative of formula II given in claim 1 in which R^1, R^2 and R^3 are as in claim 19 and A is methylene whenever prepared or produced by the process as claimed in claim 19 or an obvious chemical equivalent thereof.